SUPPLEMENTARY DATA

Supplemental Table S1. Strains and plasmids

Strains	***Description***
D282	*S. suis* serotype 2 strain isolated from pig brain, HA type P$_N$
628	*S. suis* serotype 2 strain isolated from human brain, HA type P$_N$
P1/7	*S. suis* serotype 2 strain isolated from pig meningitis, HA type P$_N$. Genomic sequence of the strain is available in Sanger Institute genomic database.
166’	*S. suis* serotype 2 strain, virulent clinical strain isolated from pig, non hemagglutinating
D282-*sadP*	*sadP*::pSF151, kan^r, single cross-over mutant
P1/7-*sadP*	*sadP*::pSF151, kan^r, single cross-over mutant
D282-*981782*	981782::pSF151, kan^r, single cross-over mutant
D282-*srtA*	*srtA*::pSF151, kan^r, single cross-over mutant
D282-Δ*dpr*	allelic replacement mutant, spc^r, [a]
D282-33	*dpr::pKUN19, spc^r, single cross-over mutant* [b]
BL21, Novablue(DE3)	*E. coli,* expression strain, Novagen
DH5a	*E. coli,* cloning strain

Plasmids	***Description***
pSadP-KO	*sadP*::pSF151, internal 433 bp PCR product (primers SadP-KO sense and SadP-KO antisense) of gene *sadP* cloned into vector pSF151
pSrtA-KO	*srtA*::pSF151, internal 573 bp PCR product (primers SRTA-5'-KO and SRTA-3'-KO) of gene *srtA* cloned into vector pSF151
pSadP	A 2.1 kb PCR product *of sadP* (primers SadP sense BamHI and SadP antisense XhoI) cloned into pET-28a
N(31-295) SadP	A 1 kb PCR product of sadP (primers SadP sense BamHI and N(31-295) cloned into pET28a
pET-28a	Novagen expression plasmid with polyhistidine and S-Tag

[a] Pulliainen, A. T., Haataja, S., Kahkonen, S., and Finne, J. (2003) *J. Biol. Chem.* **278**, 7996-8005; [b] Pulliainen, A. T., Kauko, A., Haataja, S., Papageorgiou, A. C., and Finne, J. (2005) *Mol. Microbiol.* **57**, 1086-1100; [c] Tao, L., LeBlanc, D. J., and Ferretti, J. J. (1992) *Gene* **120**, 105-110

Supplemental Table S2. Primers

SadP-KOs	TTTTTTCTGCAGATTCAACGACAACGGTTA (PstI underlined)
SadP-KOa	TTTTTTGAATTCACTTTCCACTGAATCTGA (EcoRI underlined)
SadPs	GTTGGATCCGAATCGCTAGAAC (BamHI underlined)
SadPa	TATCTCGAGTTAACTTGCTTCGCCTGTAT (XhoI underlined)
N(31-328)a	TATCTCGAGTTATTATTCTTTCTCAAGGGTAATCTC (XhoI underlined)
SadPLICs[#]	GACGACGACAAGATGAGCAAGCAGAAAGTT
SAPSeq1*	AAGGTCATTCAGGAGTTG
SAPSeq2	CTGGGAAGGAAACTTCTC
SASSeq3	TATTAACAACTCAGCCAGTC
SAPSeq4	TTATGAGCAAGCAGAAAG
SAPSeq5	GAAAGTAACTTTTGATACAAGC
SAPSeq6	GAATCCAAAACCAGAGC
T7 promoter primer	TAATACGACTCACTATAGGG
T7 terminator primer	GCTAGTTATTGCTCAGCGG
SRTA-5'-KO	TTTTTTCTGCAGCAAGAAGAAGCGTAAAGGTTC (PstI underlined)
SRTA-3'-KO:	TTTTTTGAATTCACGTAGGTGTAAACATTGGTC (EcoRI underlined)
SRTA-PROM	TGCCTTGGTAGATGCTGCCG
pSF151 MCS	TTAGCTCACTCATTAGGCAC
pSF151MCSa	TCTGTGACTGGTGAGTACTT

[#] SadPLICs, primer, that contains 5'-arm (underlined) for LIC-cloning and anneals to nt 1-18 of SadP gene, was used for verification of SadP knockout

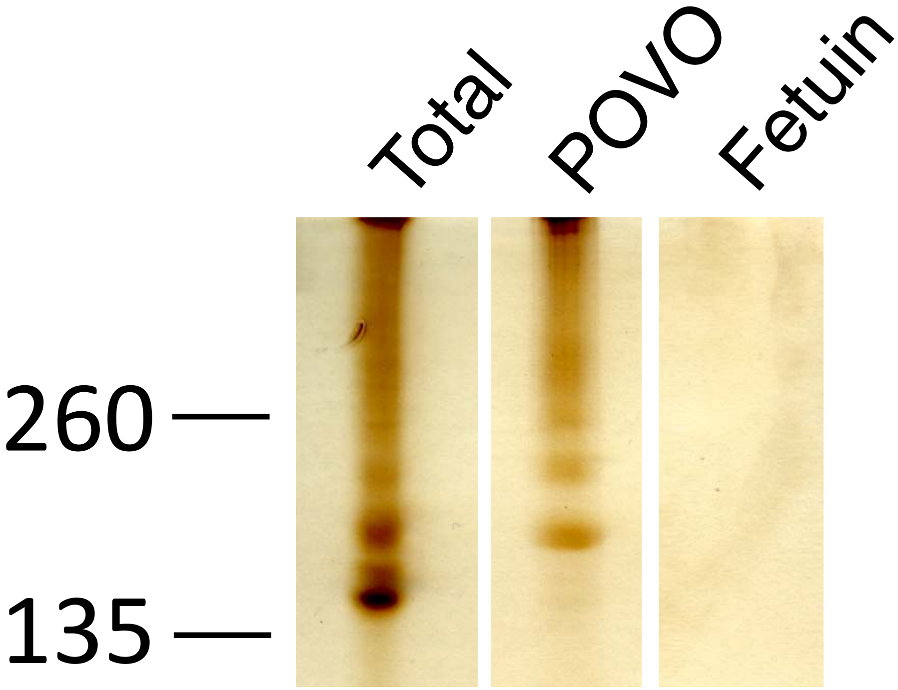
*SAPSeq1-6, sequencing primers

TABLE S3. Summary of the inhibitory values of Galα1–4Gal deoxy-derivatives obtained with recombinant SadP or whole bacteria. The inhibitory values are presented as percent binding of SadP in the presence of inhibitor compared to the binding with buffer only. Previous IC_{50} values (mM) using whole bacteria are shown for comparison.

Inhibitor	**% binding**[a]	**MIC (mM) Haataja et al.**[b]
Galα1–4Gal	5	0.091
Galα1–4Gal; 2-deoxy-	89	>2.5
Galα1–4Gal; 3-deoxy-	93	>2.5
Galα1–4Gal; 3-OMe-	124	>2.5
Galα1–4Gal; 6-deoxy-	6	0.082
Galα1–4Gal; 2'-deoxy-	7	0.058
Galα1–4Gal; 3'-deoxy-	6	0.033
Galα1–4Gal; 3'-C-Me-	9	0.30
Galα1–4Gal; 4'-deoxy-	115	>2.5
Galα1–4Gal; 6'-deoxy-	105	>2.5

[a] percent (%) binding to pigeon ovomucoid with 0.5 mM inhibitor as compared to binding with buffer only.

[b] minimum inhibitory concentrations (MIC, mM) for the inhibition of serotype 2 *S. suis* strain 628 binding to Galα1–4Galβ1–4Glcβ1–O–CETE-BSA in solid-phase inhibition assay using [^{35}S]-Methionine-labeled bacteria, Haataja, S., Tikkanen, K., Nilsson, U., Magnusson, G., Karlsson, K. A., and Finne, J. (1994) *J. Biol. Chem.* **269**, 27466-27472



Supplemental Fig. 1. **Comparison of S. suis proteins binding to pigeon ovomucoid and fetuin.** Cell wall extracts of S. suis D282 (Total) were incubated with Affi-Gel 15 matrix coupled with pigeon ovomucoid (POVO) or fetuin and the bound proteins were eluted and analyzed with SDS-PAGE and silver staining.

Supplemental Fig. 2. **The primary amino acid sequence of SadP. A.** Sequences which are excluded in the SadP fusion protein, i.e. signal peptide and the C-terminal LPNTG motif are underlined. Three highly conserved repeats are highlighted with green, and N-terminal coiled coil region predicted with Coils program is highlighted with magenta. **B.** Prediction of seven tandem repeats each 57 aa long are shown.

A.

1 mskqkvvssl llstvvlggl afystttvka eslepdvtsv nasdatnkpv vpneeggdfl

61 aeeeleddt leeelnekae evtepsspea llqprammsd setsgmeeip mndepsdnte

121 ekvekqqspl iqtsnadyks gkdqeklrts vsinllkaee gqiqwkvtfd tsewsfnvkh

181 ggvyfilpng ldltkivdnn qhditasfpt dindyrnsgq ekyrffsskq gldnengfns

241 qwnwsagqan psetvnswks gnrlskiyfi nqitdttelt ytltakvtep nqqsfpllav

301 mksftytnsk stevtslgar eitlekektl ppkenpkpep eapkpdapqa psapesptee

361 pkkedapqtp qapstpekqp evpespnpet pdapstpkde pqapsipeek pqvpeepkqe

421 apsapstpek qpeapespte epkkedapap stpekqpevp espnpetpda pstpkdepqv

481 psipeeqpke tpapeepkke dtpqtpqaps tpkeeapkee vptppapsvp eeqpketptp

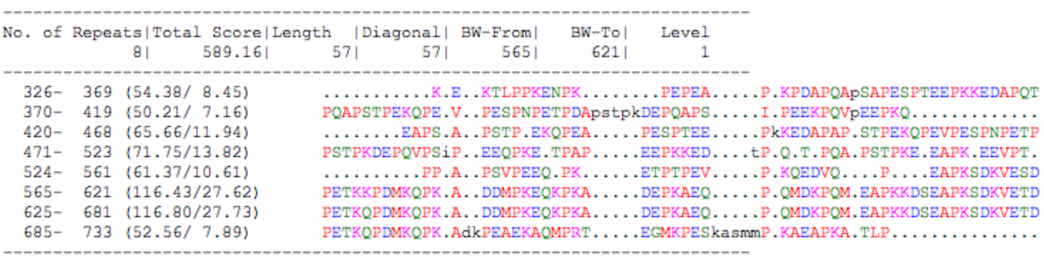
541 evpkqedvqp eapksdkves dkqmpetkkp dmkqpkaddm pkeqkpkade pkaeqpqmdk

601 pqmeapkkds eapksdkvet dkqlpetkqp dmkqpkaddm pkeqkpkade pkaeqpqmdk

661 pqmeapkkds eapksdkvet dkpmpetkqp dmkqpkadkp eaekaqmprt egmkpeskas

721 mmpkaeapka t**LPNTG**eass aigwlggala tlatglylfk nkkee

B.



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----------------------------------------------------------------------------
No. of Repeats|Total Score|Length  |Diagonal| BW-From|   BW-To|   Level
             8|     589.16|      57|      57|     565|     621|       1
----------------------------------------------------------------------------
  326-  369 (54.38/ 8.45)       ...........K.E..KTLPPKENPK........PEPEA.....P.KPDAPQApSAPESPTEEPKKEDAPQT
  370-  419 (50.21/ 7.16)       PQAPSTPEKQPE.V..PESPNPETPDApstpkDEPQAPS.....I.PEEKPQVpEEPKQ.............
  420-  468 (65.66/11.94)       ........EAPS.A..PSTP.EKQPEA.....PESPTEE.....PkKEDAPAP.STPEKQPEVPESPNPETP
  471-  523 (71.75/13.82)       PSTPKDEPQVPSiP..EEQPKE.TPAP.....EEPKKED....tP.Q.T.PQA.PSTPKE.EAPK.EEVPT.
  524-  561 (61.37/10.61)       ..........PP.A..PSVPEEQ.PK......ETPTPEV.....P.KQEDVQ....P....EAPKSDKVESD
  565-  621 (116.43/27.62)      PETKKPDMKQPK.A..DDMPKEQKPKA.....DEPKAEQ.....P.QMDKPQM.EAPKKDSEAPKSDKVETD
  625-  681 (116.80/27.73)      PETKQPDMKQPK.A..DDMPKEQKPKA.....DEPKAEQ.....P.QMDKPQM.EAPKKDSEAPKSDKVETD
  685-  733 (52.56/ 7.89)       PETKQPDMKQPK.AdkPEAEKAQMPRT.....EGMKPESkasmmP.KAEAPKA.TLP...............
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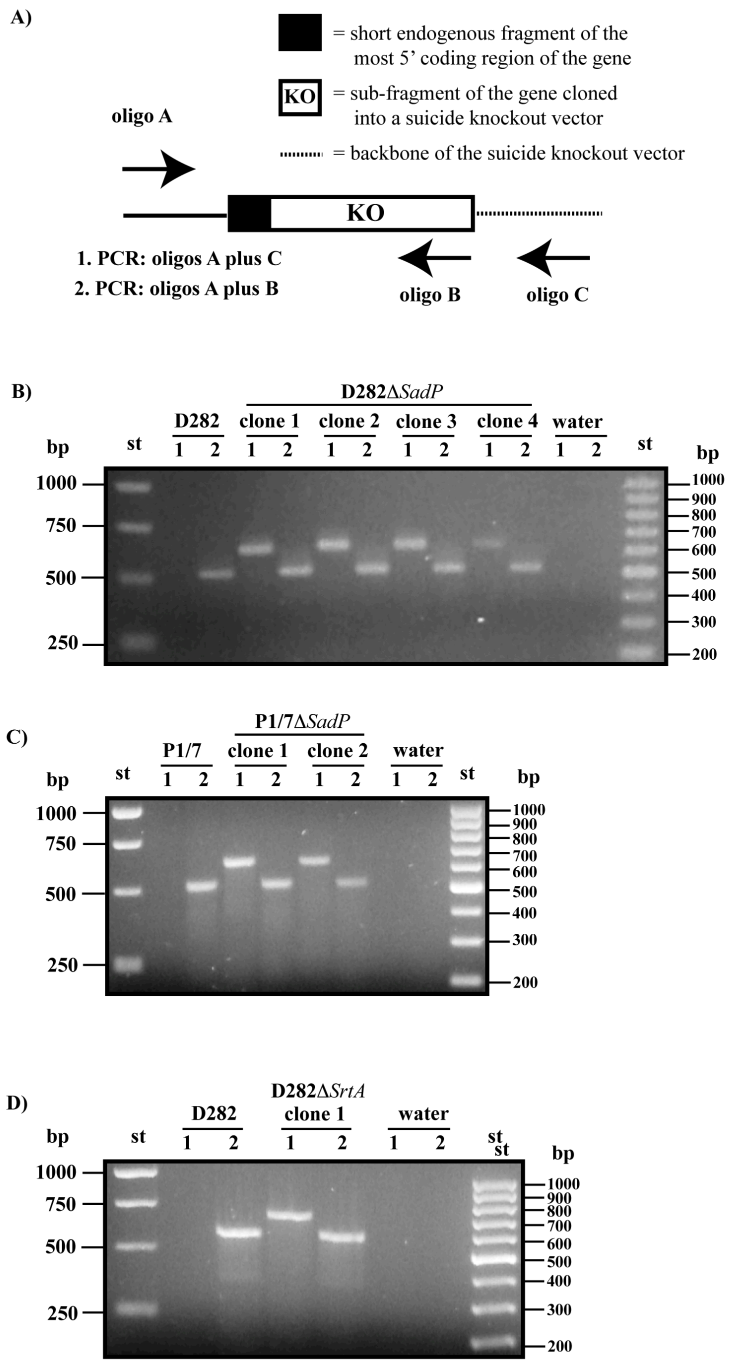
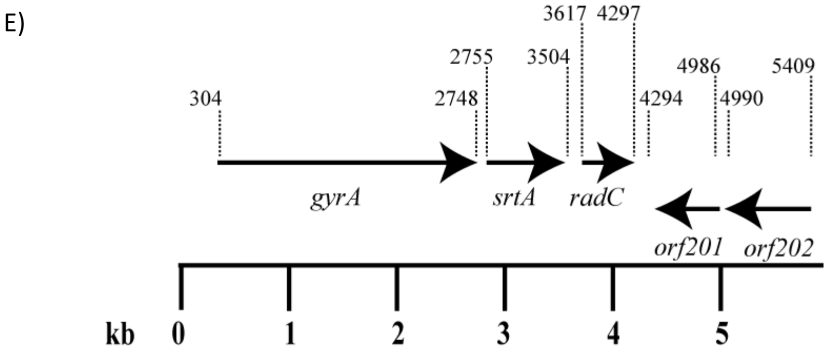
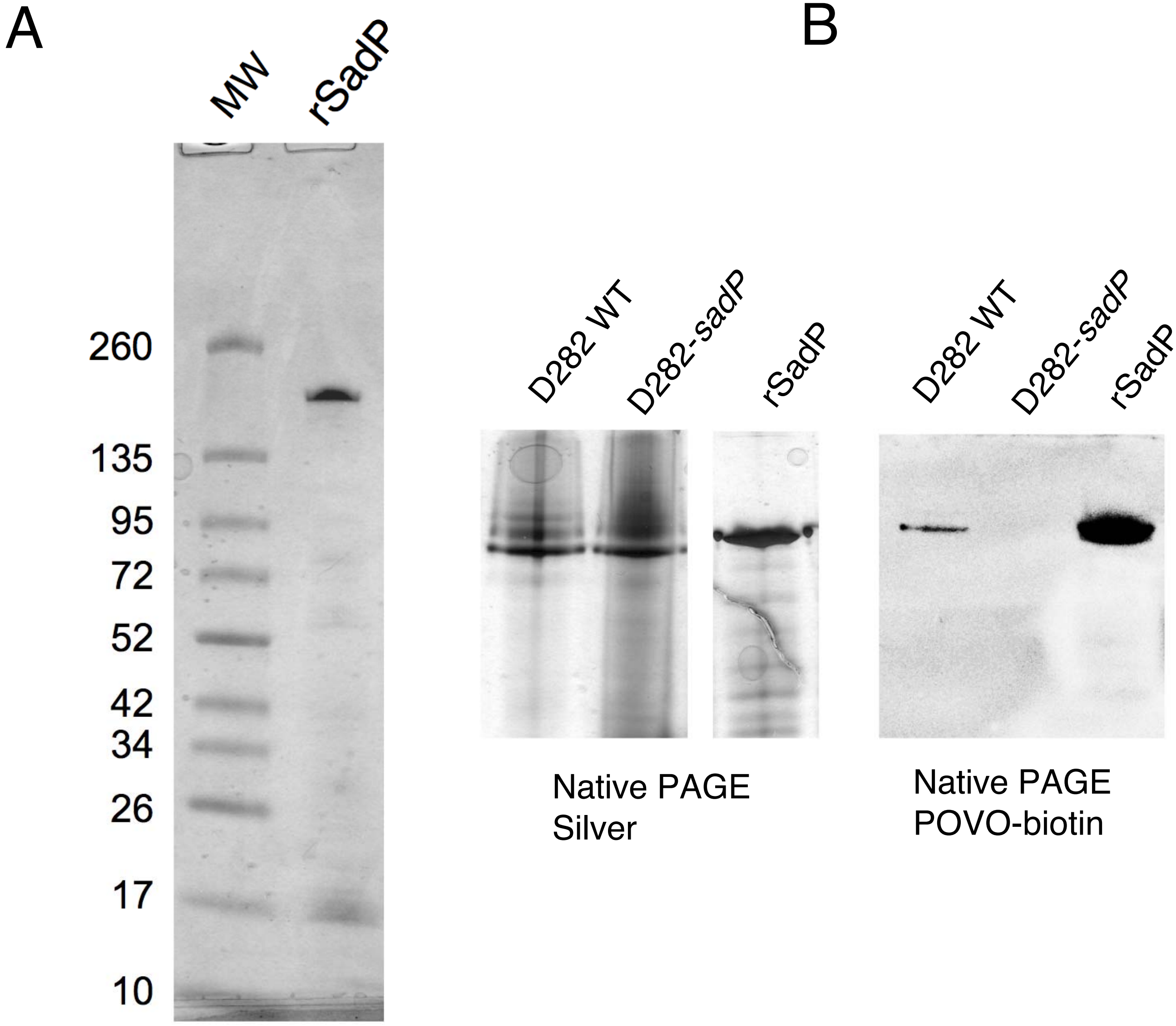




Supplemental Fig. 3. **PCR-based verification of the generated knockouts. A.** Principle of the PCR-based approach to verify insertion of the knockout plasmid into a correct gene at a correct genetic locus. This approach is based on running two independent PCR reactions. Oligos A and B amplify a fragment with wild-type and knockout whereas oligos A and C amplify only a fragment with the knockout due to insertion of the suicide vector into the correct genetic locus. **B.** Four independent SadP-knockouts were isolated in the D282 background and are here analyzed for vector insertion into the *sadP* (oligo A: SadPLICs; oligo B: SadP-KOa; oligo C: pSF151-MCS). **C.** Two independent SadP-knockouts were isolated in the P1/7 background and are here analyzed for vector insertion into the *sadP* (oligo A: SadPLICs; oligo B: SadP-KOa; oligo C: pSF151-MCS). **D.** One SrtA-knockout was isolated in the D282 background and is analyzed for vector insertion into the *srtA* (oligo A: SRTA-PROM; oligo B: SRTA-3'-KO; oligo C: pSF151-MCS). **E.** Schematic representation of the srtA-containing genomic region in *S. suis* NCTC10234 (nucleotide accession code AB066353) Osaki, M., Takamatsu, D., Shimoji, Y., and Sekizaki, T. (2002) *J. Bacteriol.* **184**, 971-982



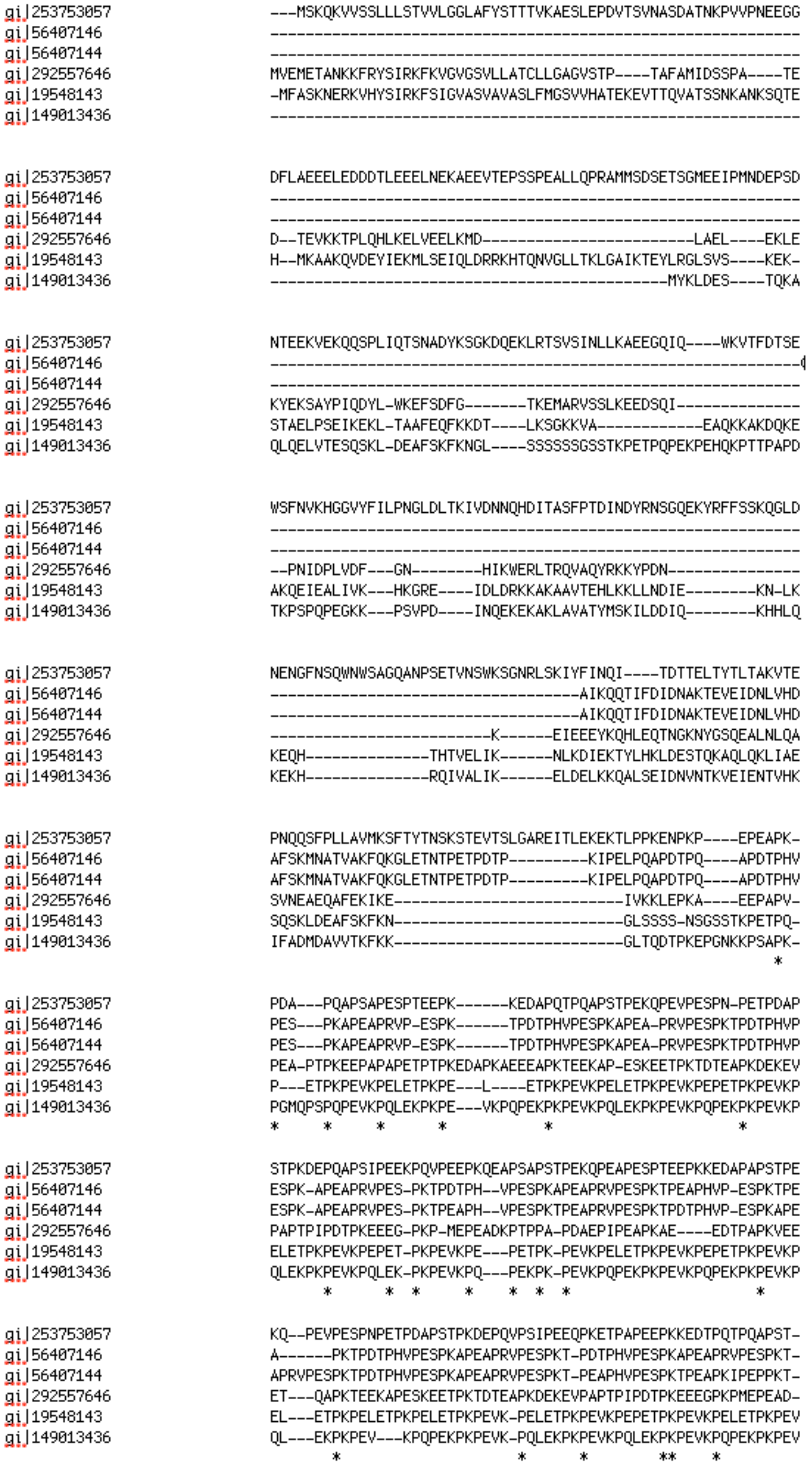
SDS-PAGE
Coomassie

Native PAGE
Silver

Native PAGE
POVO-biotin

Supplemental Fig. 4. **SDS-PAGE and non-denaturing native PAGE of recombinant and native SadP. A.** Coomassie-stained SDS-PAGE of the recombinant SadP cloned in pET28a expression vector and expressed in *E. coli*. **B.** Pigeon ovomucoid binding to lysozyme extract of wild-type D282, D282-*sadP* mutant and recombinant SadP. **The** lysozyme extracts of the bacteria and recombinant SadP (2 µg) were separated in native PAGE and the gel was stained with silver staining. A replica was blotted onto nitrocellulose membrane and probed with biotinylated pigeon ovomucoid.

Supplemental Fig. 5. **Clustal W alignment of proteins with homology to *S. suis* SadP.** The alignment shows the homology of SadP with the streptococcal proteins listed below. The conserved prolines of the tandem repeat region are marked with asterisks. There is no significant homology to SadP N-terminal sequence. Proteins included in the Clustal W comparison: gi|253753057 *S. suis* P1/7 surface protein (SadP); gi|56407146 and gi|56407144 *S. agalactiae* C protein immunoglobulin-A-binding beta antigen; gi|292557646, surface protein of *S. suis;* gi|19548143, *S. pneumoniae* PspC; gi|149013436, *S. pneumoniae* IgA-binding beta antigen.



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gi|56407146                 ------------------------------------------------------------
gi|56407144                 ------------------------------------------------------------
gi|292557646                MVEMETANKKFRYSIRKFKVGVGSVLLATCLLGAGVSTP----TAFAMIDSSPA----TE
gi|19548143                 -MFASKNERKVHYSIRKFSIGVASVAVASLFMGSVVHATEKEVTTQVATSSNKANKSQTE
gi|149013436                ------------------------------------------------------------


gi|253753057                DFLAEEELEDDDTLEEELNEKAEEVTEPSSPEALLQPRAMMSDSETSGMEEIPMNDEPSD
gi|56407146                 ------------------------------------------------------------
gi|56407144                 ------------------------------------------------------------
gi|292557646                D--TEVKKTPLQHLKELVEELKMD------------------------LAEL----EKLE
gi|19548143                 H--MKAAKQVDEYIEKMLSEIQLDRRKHTQNVGLLTKLGAIKTEYLRGLSVS----KEK-
gi|149013436                ---------------------------------------------MYKLDES----TQKA


gi|253753057                NTEEKVEKQQSPLIQTSNADYKSGKDQEKLRTSVSINLLKAEEGQIQ----WKVTFDTSE
gi|56407146                 ------------------------------------------------------------
gi|56407144                 ------------------------------------------------------------
gi|292557646                KYEKSAYPIQDYL-WKEFSDFG-------TKEMARVSSLKEEDSQI--------------
gi|19548143                 STAELPSEIKEKL-TAAFEQFKKDT----LKSGKKVA------------EAQKKAKDQKE
gi|149013436                QLQELVTESQSKL-DEAFSKFKNGL----SSSSSSGSSTKPETPQPEKPEHQKPTTPAPD


gi|253753057                WSFNVKHGGVYFILPNGLDLTKIVDNNQHDITASFPTDINDYRNSGQEKYRFFSSKQGLD
gi|56407146                 ------------------------------------------------------------
gi|56407144                 ------------------------------------------------------------
gi|292557646                --PNIDPLVDF---GN--------HIKWERLTRQVAQYRKKYPDN---------------
gi|19548143                 AKQEIEALIVK---HKGRE----IDLDRKKAKAAVTEHLKKLLNDIE--------KN-LK
gi|149013436                TKPSPQPEGKK---PSVPD----INQEKEKAKLAVATYMSKILDDIQ--------KHHLQ


gi|253753057                NENGFNSQWNWSAGQANPSETVNSWKSGNRLSKIYFINQI----TDTTELTYTLTAKVTE
gi|56407146                 -----------------------------------AIKQQTIFDIDNAKTEVEIDNLVHD
gi|56407144                 -----------------------------------AIKQQTIFDIDNAKTEVEIDNLVHD
gi|292557646                -------------------------K------EIEEEYKQHLEQTNGKNYGSQEALNLQA
gi|19548143                 KEQH--------------THTVELIK------NLKDIEKTYLHKLDESTQKAQLQKLIAE
gi|149013436                KEKH--------------RQIVALIK------ELDELKKQALSEIDNVNTKVEIENTVHK


gi|253753057                PNQQSFPLLAVMKSFTYTNSKSTEVTSLGAREITLEKEKTLPPKENPKP----EPEAPK-
gi|56407146                 AFSKMNATVAKFQKGLETNTPETPDTP---------KIPELPQAPDTPQ----APDTPHV
gi|56407144                 AFSKMNATVAKFQKGLETNTPETPDTP---------KIPELPQAPDTPQ----APDTPHV
gi|292557646                SVNEAEQAFEKIKE--------------------------IVKKLEPKA----EEPAPV-
gi|19548143                 SQSKLDEAFSKFKN--------------------------GLSSSS-NSGSSTKPETPQ-
gi|149013436                IFADMDAVVTKFKK--------------------------GLTQDTPKEPGNKKPSAPK-
                                                                                     *

gi|253753057                PDA---PQAPSAPESPTEEPK------KEDAPQTPQAPSTPEKQPEVPESPN-PETPDAP
gi|56407146                 PES---PKAPEAPRVP-ESPK------TPDTPHVPESPKAPEA-PRVPESPKTPDTPHVP
gi|56407144                 PES---PKAPEAPRVP-ESPK------TPDTPHVPESPKAPEA-PRVPESPKTPDTPHVP
gi|292557646                PEA-PTPKEEPAPAPETPTPKEDAPKAEEEAPKTEEKAP-ESKEETPKTDTEAPKDEKEV
gi|19548143                 P---ETPKPEVKPELETPKPE---L----ETPKPEVKPELETPKPEVKPEPETPKPEVKP
gi|149013436                PGMQPSPQPEVKPQLEKPKPE---VKPQPEKPKPEVKPQLEKPKPEVKPQPEKPKPEVKP
                            *     *     *      *           *                     *

gi|253753057                STPKDEPQAPSIPEEKPQVPEEPKQEAPSAPSTPEKQPEAPESPTEEPKKEDAPAPSTPE
gi|56407146                 ESPK-APEAPRVPES-PKTPDTPH--VPESPKAPEAPRVPESPKTPEAPHVP-ESPKTPE
gi|56407144                 ESPK-APEAPRVPES-PKTPEAPH--VPESPKTPEAPRVPESPKTPDTPHVP-ESPKAPE
gi|292557646                PAPTPIPDTPKEEEG-PKP-MEPEADKPTPPA-PDAEPIPEAPKAE----EDTPAPKVEE
gi|19548143                 ELETPKPEVKPEPET-PKPEVKPE---PETPK-PEVKPELETPKPEVKPEPETPKPEVKP
gi|149013436                QLEKPKPEVKPQLEK-PKPEVKPQ---PEKPK-PEVKPQPEKPKPEVKPQPEKPKPEVKP
                                  *      *  *     *    *  *  *                     *

gi|253753057                KQ--PEVPESPNPETPDAPSTPKDEPQVPSIPEEQPKETPAPEEPKKEDTPQTPQAPST-
gi|56407146                 A------PKTPDTPHVPESPKAPEAPRVPESPKT-PDTPHVPESPKAPEAPRVPESPKT-
gi|56407144                 APRVPESPKTPDTPHVPESPKAPEAPRVPESPKT-PEAPHVPESPKTPEAPKIPEPPKT-
gi|292557646                ET---QAPKTEEKAPESKEETPKTDTEAPKDEKEVPAPTPIPDTPKEEEGPKPMEPEAD-
gi|19548143                 EL---ETPKPELETPKPELETPKPEVK-PELETPKPEVKPEPETPKPEVKPELETPKPEV
gi|149013436                QL---EKPKPEV---KPQPEKPKPEVK-PQLEKPKPEVKPQLEKPKPEVKPQPEKPKPEV
                                   *                    *      *        **    *
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gi|253753057                  TKKPDMKQPKADDMPKEQ-KPKADEPKAEQPQMDKPQMEAPKKDSEAPKSDKV-----ET
gi|56407146                   STDGNTKVTVVFDKPTDADKLHLKEVTTKELA----------------------------
gi|56407144                   STDGNTKVTVVFDKPTDADKLHLKEVTTKELA----------------------------
gi|292557646                  APKDEKEVPAPTPIP--DTPKEEEGPKPMEPEADKPTPEAPKAEEEAPAPTPMPEAPKEE
gi|19548143                   TPKPELETPKPEVKPELEIPKPEVKPDNSKPQADDKKPSTPN------------NLSKDK
gi|149013436                  KPKP----------------------DNSKPQADDKKPSTTN------------NLSKDK

gi|253753057                  DKQLPETKQPDMKQPKADDMPKEQKPKADEPKAEQPQMDKPQ----------------ME
gi|56407146                   ------------------------------------------------------------
gi|56407144                   ------------------------------------------------------------
gi|292557646                  EVPAPTPAPETPMDEPKMDEPKAEMPAPAPKEEEVPTPAPMP--APETPKSEEEAPAPTP
gi|19548143                   QSSNQASTNENKKQGPATNKPKKSLPSTGSISNLALEIAGLLTLAGATILAKKRMK----
gi|149013436                  QPSNQASTNEK-----ATNKPKKSLPSTGSISNLALEIAGLLTLAGATILAKKRMK----

gi|253753057                  APKKDSEAPKSDKVETDKPMP----ETKQPDMKQPKAD---KPEAEKAQMPRTEGMKPES
gi|56407146                   ------------------------------------------------------------
gi|56407144                   ------------------------------------------------------------
gi|292557646                  MPEADKPTPEAPKAEEETPAPTPMLEDKQPEMEQPKAEDMPKEEMPKAEQPKAEDSTPKT
gi|19548143                   ------------------------------------------------------------
gi|149013436                  ------------------------------------------------------------

gi|253753057                  -------KASMMPKAE--------------------------------------------
gi|56407146                   ------------------------------------------------------------
gi|56407144                   ------------------------------------------------------------
gi|292557646                  AVPEVAPKTAEKPKLDFTTKERKVEEALPIKEEIRYDASLALGKSYLLQEGKAGKKVSVY
gi|19548143                   ------------------------------------------------------------
gi|149013436                  ------------------------------------------------------------

gi|253753057                  ----------------------------------------------------------AP
gi|56407146                   ------------------------------------------------------------
gi|56407144                   ------------------------------------------------------------
gi|292557646                  QDVIVDGKVVATNLLSETVVEGQNRILVKGSLEMKKEEVKTTPSVQSNPTLSHKGAPSAN
gi|19548143                   ------------------------------------------------------------
gi|149013436                  ------------------------------------------------------------

gi|253753057                  KATLPNTGEASSAIGWLG-GALATLATGLYLFKNKKEE---
gi|56407146                   -----------------------------------------
gi|56407144                   -----------------------------------------
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gi|19548143                   -----------------------------------------
gi|149013436                  -----------------------------------------
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